

IN THE CLAIMS

Please amend the claims as follows:

Claim 1 (Currently Amended): A method for producing a heterologous RNA of interest, ~~which the method is characterized in that it comprises at least the following steps~~ comprising:

(1) transforming the mitochondria of yeast cells lacking mitochondrial DNA with a mitochondrial transcription vector ~~comprising that comprises~~ comprising at least one copy of the DNA encoding said heterologous RNA of interest under the control of regulatory element(s) for mitochondrial transcription, and a mitochondrial transformation reporter gene or a fragment of said reporter gene;

(2) identifying the yeast mitochondrial transformants that have incorporated the DNA of interest;

(3) culturing the yeast mitochondrial transformants selected in step (2);

(4) isolating the mitochondria from the yeast mitochondrial transformants obtained in ~~step~~ (3), and

(5) extracting and purifying the heterologous RNA of interest from said mitochondria.

Claim 2 (Currently Amended): The method as claimed in claim 1, ~~characterized in that~~ wherein said yeast cells lacking mitochondrial DNA are *rho*⁰ cells.

Claim 3 (Currently Amended): The method as claimed in claim 1, wherein ~~or claim 2, characterized in that~~ said cells lacking mitochondrial DNA are obtained from a Δ *SUV3* or Δ *DSS1* strain.

Claim 4 (Currently Amended): The method as claimed in ~~any one of claims 1 to 3,~~
~~characterized in that~~ claim 1, wherein said cells lacking mitochondrial DNA comprise a
chromosomal copy of a gene encoding an exogenous RNA polymerase and including
includes a mitochondrial targeting signal.

Claim 5 (Currently Amended): The method as claimed in ~~any one of claims 1 to 4,~~
~~characterized in that~~ claim 1, wherein said DNA encoding the RNA of interest is under the
control of a promoter and a transcription terminator that are functional in yeast mitochondria.

Claim 6 (Currently Amended): The method as claimed in ~~any one of claims 1 to 5,~~
~~characterized in that~~ claim 1, wherein said mitochondrial transformation reporter gene is a
gene encoding one of the proteins of a yeast respiratory chain.

Claim 7 (Currently Amended): The method as claimed in ~~any one of claims 1 to 6,~~
~~characterized in that~~ claim 1, wherein said mitochondrial transcription vector comprises the
sequence of an origin of replication of the mitochondrial DNA.

Claim 8 (Currently Amended): The method as claimed in ~~any one of claims 1 to 7,~~
~~characterized in that~~ claim 1, wherein the transformation according to ~~step~~ (1) comprises the
adsorption of said mitochondrial transcription vector onto metal microprojectiles and the
projection of said microprojectiles onto said cells.

Claim 9 (Currently Amended): The method as claimed in ~~any one of claims 1 to 8,~~
~~characterized in that~~ claim 1, wherein ~~step~~ (1) comprises the cotransformation of said yeast

cells with said mitochondrial transcription vector and a vector that is replicative in yeast, comprising a nuclear selection marker.

Claim 10 (Currently Amended): The method as claimed in claim 9, ~~characterized in that wherein~~ said nuclear marker is an auxotrophic marker of said transformed cells.

Claim 11 (Currently Amended): The method as claimed in ~~any one of claims 1 to 10, characterized in that step~~ claim 1, wherein (2) comprises:

(a₀) crossing the yeast mitochondrial transformants obtained in step (1) with a yeast tester strain of *rho*⁺ mit⁻ type,

(b₀) identifying the mitochondrial transformants which, once crossed, give diploid cells capable of growing on a non-fermentable medium, and

(c₀) repeating said crossing until isolated yeast colonies identified as being mitochondrial transformants carrying the mitochondrial transformation vector are obtained.

Claim 12 (Currently Amended): The method as claimed in claim 9, ~~wherein or claim 10, characterized in that step~~ (2) comprises:

(a₁) a first selection or preselection of the yeast cells by means of said nuclear marker, by culturing in an appropriate medium, and

(b₁) a second selection from the yeast cells selected in (a₁), in accordance with steps (a₀), (b₀) and (c₀), as defined in claim 11.

Claim 13 (Currently Amended): The method as claimed in ~~any one of claims 1 to 12, characterized in that~~ claim 1, wherein the isolation of the mitochondria, in accordance with step (4) of the method, comprises lysis or grinding of said cells, and then at least two

centrifugation steps, at speeds preferably of between 750 g and 12,500 g, and recovery of the final centrifugation pellet.

Claim 14 (Currently Amended): The method as claimed in ~~any one of claims 1 to 13,~~
~~characterized in that~~ claim 1, wherein step (5) advantageously comprises:

- eliminating the contaminating nucleic acids in the presence of appropriate buffers, the first buffer comprising at least one divalent ion-chelating agent, and the second buffer comprising an RNase and, optionally, a DNase,
- lysing the mitochondria in the presence of at least one detergent and a divalent ion-chelating agent and within a pH range of between 7 and 8, and
- isolating and purifying the RNA of interest.

Claim 15 (Currently Amended): A modified yeast cell, ~~characterized in that it~~ which lacks mitochondrial DNA and ~~in that it comprises~~ that comprises a chromosomal copy of a gene encoding an exogenous RNA polymerase and including a mitochondrial targeting signal.

Claim 16 (Currently Amended): A modified yeast cell, ~~characterized in that it~~ which lacks mitochondrial DNA and ~~in that it's~~ the mitochondria are transformed with a mitochondrial transcription vector as defined in ~~claims 1 and 5 to 7~~ claim 1.

Claim 17 (Currently Amended): The modified yeast cell ~~as claimed in~~ of claim 15, ~~which or claim 16, characterized in that it~~ is obtained from a $\Delta SUV3$ or $\Delta DSS1$ strain.

Claim 18 (Currently Amended): The modified yeast cell ~~as claimed in any one of claims 15 to 17, characterized in that it~~ of claim 15, which is obtained from a ρ^0 strain.

Claim 19 (Canceled).

Claim 20 (Currently Amended): A system for carrying out the industrial production of a heterologous RNA of interest, ~~characterized in that it comprises~~ the system comprising:

- yeast cells lacking mitochondrial DNA, ~~in particular of ρ^0 strain,~~ transformed with at least one mitochondrial transcription vector as defined in ~~claims 1 to 5 and 7~~ claim 1,
- at least one suitable culture medium for selecting said transformed cells,
- yeast tester cells of ρ^+ mit⁻ type,
- appropriate fermenters and culture media, and
- appropriate reagents for isolating the mitochondria from synthetic ρ^- cells and extracting the heterologous RNA of interest ~~therefrom~~.